P. ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)	
31 October 2000 (31.10.00)	
International application No.	**Applicant's or agent's file reference**
PCT/EP00/02048	FB/BC45225
International filing date (day/month/year)	**Priority date** (day/month/year)
09 March 2000 (09.03.00)	11 March 1999 (11.03.99)
Applicant	
BRUCK, Claudine, Elvire, Marie et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

21 September 2000 (21.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
SMITHKLINE BEECHAM PLC
Attn. Dalton, Marcus Jonathan
Two New Horizons Court
Brentford
Middlesex TW8 9EP
UNITED KINGDOM

	Date of mailing (day/month/year) 02/10/2000
Applicant's or agent's file reference FB/BC45225	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/EP 00/ 02048	International filing date (day/month/year) 09/03/2000
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Renate Jordan

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1 [Where originally there were 48 claims and after amendment of some claims there are 51]
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2 [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3 [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4 [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14, claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1))

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
FB/BC45225		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 02048	09/03/2000	11/03/1999
Applicant		
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   CASB618 POLYNUCLEOTIDES AND POLYPEPTIDES AND THEIR USE

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02048

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C12N15/75 C12N5/10 A61K38/17
C07K16/18 G01N33/68 G01N33/574 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"ql49c11.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1875668 3' ....." EMBL SEQUENCE DATABASE, 23 November 1998 (1998-11-23), XP002147269 HEIDELBERG DE Ac AI274929 the whole document ---	5,8,12, 13
X	"qk58e08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1873190 3' ....." EMBL SEQUENCE DATABASE, 24 November 1998 (1998-11-24), XP002147270 HEIDELBERG DE Ac AI281211 the whole document ---	5,8,12, 13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2000	02/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 32865 A (INCYTE PHARMA INC ;GOLI SURYA K (US); HILLMAN JENNIFER L (US)) 30 July 1998 (1998-07-30) abstract	14-16, 22,23, 28-31
A	WO 96 08514 A (ARLEN MYRON ;TSANG KWONG Y (US)) 21 March 1996 (1996-03-21) abstract; claims	17,22,28
P,A	WO 99 49030 A (BRUCK CLAUDINE ELVIRE MARIE ;SMITHKLINE BEECHAM BIOLOG (BE); COCHE) 30 September 1999 (1999-09-30) the whole document	1-25, 28-33
P,A	WO 99 49055 A (BRUCK CLAUDINE ELVIRE MARIE ;SMITHKLINE BEECHAM BIOLOG (BE); COCHE) 30 September 1999 (1999-09-30) the whole document	1-25, 28-33

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 24 and 25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

Claims Nos.: 26 27

Claim 26 and claim 27 (as far as it refers to (ant)agonists of the polypeptide of claims 1-5) refer to agonists/antagonists of the polypeptide of claims 1-5 without giving a true technical characterization. in consequence the scope of said claims is ambigous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/02048

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 26 27
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/02048

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9832865 A	30-07-1998	US 5840871 A	24-11-1998
		AU 6041998 A	18-08-1998
		EP 0975769 A	02-02-2000
WO 9608514 A	21-03-1996	US 5688657 A	18-11-1997
		AU 3587095 A	29-03-1996
		CA 2199740 A	21-03-1996
		EP 0777690 A	11-06-1997
		JP 10505749 T	09-06-1998
		ZA 9507634 A	27-05-1996
WO 9949030 A	30-09-1999	AU 3519199 A	18-10-1999
WO 9949055 A	30-09-1999	AU 3519299 A	18-10-1999

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

GIDDINGS, Peter John
GLAXOSMITHKLINE
Corporate Intellectual Property
Two New Horizons Court
Brentford
Middlesex TW8 9EP
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.06.2001

Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
SD/FB/BC45225	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02048	09/03/2000	11/03/1999

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Büchler, S Tel.+49 89 2399-8090

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SD/FB/BC45225		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02048	09/03/2000	11/03/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/09/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Valcarcel, R Telephone No. +49 89 2399 2368

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02048

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-48	as originally filed

Claims, No.:

1-31	as received on	28/05/2001	with letter of	25/05/2001
32-34	with telefax of	18/06/2001		

Drawings, sheets:

1/7-7/7	as originally filed

Sequence listing part of the description, pages:

1-14, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence

listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 27,28; 25,26 (with respect to industrial applicability).

because:

   ☒ the said international application, or the said claims Nos. 25,26 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 27,28.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4,6,7,9-11,18-26,29-34
	No:	Claims	5,8,12-17
Inventive step (IS)	Yes:	Claims	1-4,6,7,9-11,18-26,29-34
	No:	Claims	5,8,12-17
Industrial applicability (IA)	Yes:	Claims	1-24,29-34
	No:	Claims	NONE

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

The priority document filed on 11.03.1999, discloses only one polynucleotide sequence, the one of SEQ ID NO: 1, which corresponds to the SEQ ID NO: 3 of the present application. This SEQ ID NO: 1 of the first priority document (or SEQ ID NO: 3 of the present application) corresponds to a fragment of the SEQ ID NO: 1 of the present application (and it is an EST that corresponds to a partial cDNA). Furthermore, this priority document dated on 11.03.1999 does not disclose any amino acid sequence (although it makes reference in the description to a polypeptide having the amino acid sequence as set forth in SEQ ID NO: 2). Thus, only the claim referring to SEQ ID NO: 3 of the present application (claim 32) is entitled the priority right of the 11.03.1999.

The second priority document, filed on 01.09.1999 discloses SEQ ID NOs: 1 to 3. Thus, the international application **WO 00 56891**, could become relevant in the Regional Phase (as for example in front of the EPO) since it has an earlier priority 22.03.1999 (see Item VII of the present communication).

Re Item III

1. **The subject-matter of claims 27 and 28 has not been searched**. According to Rule 66.1(e) PCT, **the IPEA has not carried out examination** on non-searched subject-matter.

2. **Claims 25 and 26** relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Claims 24 and 25 relate to methods of treatment of the human or animal body by therapy. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

1. Reference is made to the following documents; the numbering corresponds to the order of citation in the International Search Report (ISR).

   D1: 'ql49c11.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1875668 3' .....' EMBL SEQUENCE DATABASE, 23 November 1998 (1998-11-23), HEIDELBERG DE. Accession Number **AI274929**.

   D2: 'qk58e08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1873190 3' .....' EMBL SEQUENCE DATABASE, 24 November 1998 (1998-11-24), HEIDELBERG DE. Accession Number **AI281211**.

2. The present application does not satisfy the criterion set forth in Article 33(2) PCT because **the subject-matter of claims 5, 8, 12 to 17 is not new** in respect of prior art as defined in the regulations (Rule 64 PCT).

   D1 discloses a nucleotide sequence of 405 nucleotides which shows 100 % identity to the nucleotides 1009 to 1413 of SEQ ID NO: 1 of the present application. The nucleotide sequence of D1 encodes for a polypeptide (of 70 amino acids) which sequence is identical to the sequence of amino acids 251 to 320 of SEQ ID NO: 2 of the present application.

   D2 discloses a nucleotide sequence of 381 nucleotides which shows 100 % identity to the nucleotides 1033 to 1413 of SEQ ID NO: 1 of the present application. The nucleotide sequence of D1 encodes for a polypeptide (of 62 amino acids) which sequence is identical to the sequence of amino acids 259 to 320 of SEQ ID NO: 2 of the present application.

   Thus both D1 and D2 disclose a polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 said immunogenic fragment being capable of raising an immune response which recognises the polypeptide of SEQ ID NO: 2. **Thus D1 and D2 are prejudicial to the novelty of claim 5.**

Accordingly, **D1 and D2 are also prejudicial to the novelty of claim 8**, since D1 and D2 disclose isolated polynucleotides encoding a polypeptide as claimed in claim 5. **D1 and D2 are also prejudicial to the novelty of claim 12**, since D1 and D2 disclose isolated polynucleotides encoding as defined in claim 8 in which the identity is at least 95 %. **D1 and D2 are also prejudicial to the novelty of claim 13**, since D1 and D2 disclose isolated polynucleotides obtainable by screening an appropriate library as defined in claim 13 (c). It is noted that there is no specific disclosure in D1 and D2 of any immunogenic properties associated with the ESTs disclosed, however, the immunogenic properties of the proteins encoded by such ESTs are intrinsic features of the proteins, and thus, even if not disclosed, D1 and D2 are prejudicial to the novelty of these claims.

D1 and D2 further disclose expression vectors according to claim 14. Such vectors are kept in recombinant bacteria which fall under the scope of claims 15 and 16, and such vectors are used in processes to express peptides, it is known by an skilled person in the art that EST libraries express the ESTs. Thus **D1 and D2 are also prejudicial to the novelty of claims 14 to 17.**

3. **The subject-matter of claims 1 to 4, 6, 7, 9 to 11 involves an inventive step.**

The underlying technical problem would have been to find new sequences which could be used in the treatment of cancer (and in particular in the treatment of colon cancer). A person skilled in the art in search of such sequences would have looked for ESTs which are expressed in colon.

D1 and D2 disclose the sequences of two ESTs (D2 is a fragment of D1) which are disclosed **to be expressed in colon** (see the section giving information about the source: it is specified that the source organ was colon). A person skilled in the art could have consulted an EST database to check how many ESTs corresponding to the same cDNA as D1 and D2 are expressed in colon tumours, and could have seen that there was a high proportion of colon cancer ESTs corresponding to the EST of D1. Both D1 and D2 give an INTERNET address (http://www.ncbi.nlm.nih.gov/ncicgap) wherein the skilled person could consult the public EST database. It appears that the EST database used in example 3 of the

present application was also a public database.

Having a partial EST sequence, the isolation of the complete cDNA sequence corresponding to this EST would not have involved an inventive step since the skilled person would know of methods to isolate this sequence without the exercise of inventive skill.

However, in view of the arguments exposed in the Applicant's letter of reply dated on 25 May 2001, in particular, in view of the large number of ESTs derived from colon cancer libraries, it appears that the skilled person would not have been able to select the sequences of the present application without further experimentation among other EST expressed in colon cancer, and thus the identification of such sequences and the use of such sequences in methods of treatment appear to involve an inventive step.

4. Accordingly, claims 18 to 26, 29 to 34 also appear to meet the requirements of the PCT in respect of inventive step.

5. For the assessment of the present claims 25 and 26 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The EPO does not recognize as industrially applicable methods of treatment of the human body by surgery or therapy and diagnostic methods practised on the human or animal body. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 00 56891	28 September 2000	22 March 2000	22 March 1999

SEQ ID NO: 57 of WO 00 56891 has 100% identity in 1413 overlap with SEQ ID NO: 1 of the present application (which has 1441 nt). SEQ ID NO: 57 of WO 00 56891 has 99,6% identity in 477 overlap with SEQ ID NO: 3 of the present application (which has 498 nt). And SEQ ID NO: 28 of WO 00 56891 is identical to SEQ ID NO: 2 of the present application.

Re Item VIII

Claim 13 is not clear since it refers to an isolated polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions. It is unclear which **"stringent" conditions** are referred to. According to the PCT Preliminary Examination Guidelines, the meaning of the terms of a claim should, as far as possible, be clear for the person skilled in the art **from the wording of the claim alone**. "Each claim should be studied by the examiner giving the words the meaning and scope which they normally have in the relevant art, unless in particular cases the description gives the words a special meaning, by explicit definition or otherwise. Moreover, if such a special meaning applies, the examiner should, as far as possible, **require the claim to be amended whereby the meaning is clear from the wording of the claim alone**" (see Guidelines, Chapter III, Section 4.2).

Claims

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to SEQ ID NO:2.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence of SEQ ID NO:2.

4. The isolated polypeptide of SEQ ID NO:2.

5. A polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 said immunogenic fragment being capable of raising an immune response which recognises the polypeptide of SEQ ID NO:2.

6. A polypeptide as claimed in any of claims 1 to 5 wherein said polypeptide is part of a larger fusion protein.

7. A polypeptide as claimed in any of claims 1 to 6 chemically conjugated to a carrier protein.

8. An isolated polynucleotide encoding a polypeptide as claimed in any of claims 1 to 6.

9. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

10. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

11. An isolated polynucleotide which comprises a nucleotide sequence which has at least 70% identity to that of SEQ ID NO:1 over the entire length of SEQ ID NO:1; or a nucleotide sequence complementary to said isolated polynucleotide.

12. The isolated polynucleotide as defined in any one of claims 8 to 11 in which the identity is at least 95%.

13. An isolated polynucleotide selected from:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(b) the polynucleotide of SEQ ID NO:1; and

(c) a polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof said polynucleotide encoding a protein which has similar immunogenic properties to those of the protein of sequence ID NO:2

or a nucleotide sequence complementary to said isolated polynucleotide

14. An expression vector comprising an isolated polynucleotide according to any one of claims 8-13.

15. A recombinant live microorganism comprising the expression vector of claim 14.

16. A host cell comprising the expression vector of claim 14 or the isolated polynucleotide of claims 8 to 13.

17. A process for producing a polypeptide of claims 1 to 7 comprising culturing a host cell of claim 16 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

18. A vaccine comprising an effective amount of the polypeptide of any one of claims 1 to 7 and a pharmaceutically acceptable carrier.

19. A vaccine comprising an effective amount of the polynucleotide of any one of claims 8 to 13 and a pharmaceutically effective carrier.

20. A vaccine comprising an effective amount of antigen presenting cells, modified by in vitro loading with a polypeptide of any one of claims 1 to 7, or genetically modified in vitro to express a polypeptide of claims 1 to 7 and a pharmaceutically effective carrier.

21. A vaccine as claimed in any one of claims 18 to 20 which additionally comprises a TH-1 inducing adjuvant.

22. A vaccine as claimed in claim 21 in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

23. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

24. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of any one of claims 1 to 5 which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the said polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any one of claims 1 to 7, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

25. A method for the treatment of a subject by immunoprophylaxis or therapy comprising *in vitro* induction of immune responses to a molecule of any one of claims 1 to 5, using *in vitro* incubation of the polypeptide of any one of claims 1 to 7 or the polynucleotide of any one of claims 8 to 13 with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for the treatment of disease.

26. A method as claimed in claim 25 wherein the treatment is for ovarian or colon cancer.

27. An agonist or antagonist to the polypeptide of claims 1 to 5.

28. A compound which is:
(a) an agonist or antagonist to the polypeptide of claims 1 to 5;
(b) isolated polynucleotide of claims 8 to 13; or
(c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of any one of claims 1 to 5;
for use in therapy.

29. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

30. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

31. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

32. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

33. An isolated polynucleotide selected from the group consisting of:
(a) an isolated polynucleotide comprising a nucleotide sequence which has at least 90% identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;
(b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3;
(c) the polynucleotide of SEQ ID NO:3.

34. A live vaccine composition comprising an expression vector according to claim 14 or recombinant live micro-organism according to claim 15.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SD/FB/BC45225		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02048	09/03/2000	11/03/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/09/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Valcarcel, R Telephone No. +49 89 2399 2368

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02048

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-48	as originally filed			

Claims, No.:

1-31	as received on	28/05/2001	with letter of	25/05/2001
32-34	with telefax of	18/06/2001		

Drawings, sheets:

1/7-7/7	as originally filed

Sequence listing part of the description, pages:

1-14, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.
   ☒ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence

listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 27,28; 25,26 (with respect to industrial applicability).

because:

   ☒ the said international application, or the said claims Nos. 25,26 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 27,28.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4,6,7,9-11,18-26,29-34
	No:	Claims	5,8,12-17
Inventive step (IS)	Yes:	Claims	1-4,6,7,9-11,18-26,29-34
	No:	Claims	5,8,12-17
Industrial applicability (IA)	Yes:	Claims	1-24,29-34
	No:	Claims	NONE

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II

The priority document filed on 11.03.1999, discloses only one polynucleotide sequence, the one of SEQ ID NO: 1, which corresponds to the SEQ ID NO: 3 of the present application. This SEQ ID NO: 1 of the first priority document (or SEQ ID NO: 3 of the present application) corresponds to a fragment of the SEQ ID NO: 1 of the present application (and it is an EST that corresponds to a partial cDNA). Furthermore, this priority document dated on 11.03.1999 does not disclose any amino acid sequence (although it makes reference in the description to a polypeptide having the amino acid sequence as set forth in SEQ ID NO: 2). Thus, only the claim referring to SEQ ID NO: 3 of the present application (claim 32) is entitled the priority right of the 11.03.1999.

The second priority document, filed on 01.09.1999 discloses SEQ ID NOs: 1 to 3. Thus, the international application **WO 00 56891**, could become relevant in the Regional Phase (as for example in front of the EPO) since it has an earlier priority 22.03.1999 (see Item VII of the present communication).

Re Item III

1. **The subject-matter of claims 27 and 28 has not been searched**. According to Rule 66.1(e) PCT, **the IPEA has not carried out examination** on non-searched subject-matter.

2. **Claims 25 and 26** relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Claims 24 and 25 relate to methods of treatment of the human or animal body by therapy. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

1. Reference is made to the following documents; the numbering corresponds to the order of citation in the International Search Report (ISR).

   D1: 'ql49c11.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1875668 3' .....' EMBL SEQUENCE DATABASE, 23 November 1998 (1998-11-23), HEIDELBERG DE. Accession Number **AI274929**.

   D2: 'qk58e08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1873190 3' .....' EMBL SEQUENCE DATABASE, 24 November 1998 (1998-11-24), HEIDELBERG DE. Accession Number **AI281211**.

2. The present application does not satisfy the criterion set forth in Article 33(2) PCT because **the subject-matter of claims 5, 8, 12 to 17 is not new** in respect of prior art as defined in the regulations (Rule 64 PCT).

   D1 discloses a nucleotide sequence of 405 nucleotides which shows 100 % identity to the nucleotides 1009 to 1413 of SEQ ID NO: 1 of the present application. The nucleotide sequence of D1 encodes for a polypeptide (of 70 amino acids) which sequence is identical to the sequence of amino acids 251 to 320 of SEQ ID NO: 2 of the present application.

   D2 discloses a nucleotide sequence of 381 nucleotides which shows 100 % identity to the nucleotides 1033 to 1413 of SEQ ID NO: 1 of the present application. The nucleotide sequence of D1 encodes for a polypeptide (of 62 amino acids) which sequence is identical to the sequence of amino acids 259 to 320 of SEQ ID NO: 2 of the present application.

   Thus both D1 and D2 disclose a polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 said immunogenic fragment being capable of raising an immune response which recognises the polypeptide of SEQ ID NO: 2. **Thus D1 and D2 are prejudicial to the novelty of claim 5.**

Accordingly, **D1 and D2 are also prejudicial to the novelty of claim 8**, since D1 and D2 disclose isolated polynucleotides encoding a polypeptide as claimed in claim 5. **D1 and D2 are also prejudicial to the novelty of claim 12**, since D1 and D2 disclose isolated polynucleotides encoding as defined in claim 8 in which the identity is at least 95 %. **D1 and D2 are also prejudicial to the novelty of claim 13**, since D1 and D2 disclose isolated polynucleotides obtainable by screening an appropriate library as defined in claim 13 (c). It is noted that there is no specific disclosure in D1 and D2 of any immunogenic properties associated with the ESTs disclosed, however, the immunogenic properties of the proteins encoded by such ESTs are intrinsic features of the proteins, and thus, even if not disclosed, D1 and D2 are prejudicial to the novelty of these claims.

D1 and D2 further disclose expression vectors according to claim 14. Such vectors are kept in recombinant bacteria which fall under the scope of claims 15 and 16, and such vectors are used in processes to express peptides, it is known by an skilled person in the art that EST libraries express the ESTs. Thus **D1 and D2 are also prejudicial to the novelty of claims 14 to 17.**

3. **The subject-matter of claims 1 to 4, 6, 7, 9 to 11 involves an inventive step.**

The underlying technical problem would have been to find new sequences which could be used in the treatment of cancer (and in particular in the treatment of colon cancer). A person skilled in the art in search of such sequences would have looked for ESTs which are expressed in colon.

D1 and D2 disclose the sequences of two ESTs (D2 is a fragment of D1) which are disclosed **to be expressed in colon** (see the section giving information about the source: it is specified that the source organ was colon). A person skilled in the art could have consulted an EST database to check how many ESTs corresponding to the same cDNA as D1 and D2 are expressed in colon tumours, and could have seen that there was a high proportion of colon cancer ESTs corresponding to the EST of D1. Both D1 and D2 give an INTERNET address (http://www.ncbi.nlm.nih.gov/ncicgap) wherein the skilled person could consult the public EST database. It appears that the EST database used in example 3 of the

present application was also a public database.

Having a partial EST sequence, the isolation of the complete cDNA sequence corresponding to this EST would not have involved an inventive step since the skilled person would know of methods to isolate this sequence without the exercise of inventive skill.

However, in view of the arguments exposed in the Applicant's letter of reply dated on 25 May 2001, in particular, in view of the large number of ESTs derived from colon cancer libraries, it appears that the skilled person would not have been able to select the sequences of the present application without further experimentation among other EST expressed in colon cancer, and thus the identification of such sequences and the use of such sequences in methods of treatment appear to involve an inventive step.

4. Accordingly, claims 18 to 26, 29 to 34 also appear to meet the requirements of the PCT in respect of inventive step.

5. For the assessment of the present claims 25 and 26 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The EPO does not recognize as industrially applicable methods of treatment of the human body by surgery or therapy and diagnostic methods practised on the human or animal body. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VI

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 00 56891	28 September 2000	22 March 2000	22 March 1999

SEQ ID NO: 57 of WO 00 56891 has 100% identity in 1413 overlap with SEQ ID NO: 1 of the present application (which has 1441 nt). SEQ ID NO: 57 of WO 00 56891 has 99,6% identity in 477 overlap with SEQ ID NO: 3 of the present application (which has 498 nt). And SEQ ID NO: 28 of WO 00 56891 is identical to SEQ ID NO: 2 of the present application.

Re Item VIII

Claim 13 is not clear since it refers to an isolated polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions. It is unclear which **"stringent" conditions** are referred to. According to the PCT Preliminary Examination Guidelines, the meaning of the terms of a claim should, as far as possible, be clear for the person skilled in the art **from the wording of the claim alone**. "Each claim should be studied by the examiner giving the words the meaning and scope which they normally have in the relevant art, unless in particular cases the description gives the words a special meaning, by explicit definition or otherwise. Moreover, if such a special meaning applies, the examiner should, as far as possible, **require the claim to be amended whereby the meaning is clear from the wording of the claim alone**" (see Guidelines, Chapter III, Section 4.2).

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, C07K 14/47, C12N 15/75, 5/10, A61K 38/17, C07K 16/18, G01N 33/68, 33/574, C12Q 1/68

A2

(11) International Publication Number: WO 00/53748

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(57) Abstract

CASB618 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing CASB618 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly ovarian and colon cancers, autoimmune diseases, and related conditions.

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Novel Compounds

The present invention relates to polynucleotides, herein referred to as CASB618 polynucleotides, polypeptides encoded thereby (referred to herein as CASB618 polypeptides), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including the treatment of cancer in particular in the treatment of colon cancer, and autoimmune diseases and other related conditions. In a further aspect, the invention relates to methods for identifying agonists and antagonists/inhibitors using the materials provided by the invention, and treating conditions associated with CASB618 polypeptide imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate CASB618 polypeptide activity or levels.

Polypeptides and polynucleotides of the present invention are important immunogens for specific prophylactic or therapeutic immunization against tumours, because they are specifically expressed or highly over-expressed in tumours compared to normal cells and can be targeted by antigen-specific immune mechanisms leading to the destruction of the tumour cell. They can also be used to diagnose the occurrence of tumour cells. Furthermore, their inappropriate expression in certain circumstances can cause an induction of autoimmune, inappropriate immune responses, which can be corrected through appropriate vaccination using the same polypeptides or polynucleotides. In this respect the most important biological properties are the antigenic and immunogenic activities of the polypeptide of the present invention. A polypeptide of the present invention may also exhibit at least one other biological activity of a CASB618 polypeptide, which could qualify it as a target for therapeutic or prophylactic intervention different from that linked to its use as an immunotherapeutic.

Functional genomics relies heavily on high-throughput DNA sequencing technologies and the various tools of bioinformatics to identify gene sequences of potential interest from the many molecular biology databases now available. cDNA libraries enriched for genes of relevance to a particular tissue or physiological situation can be constructed using recently developed subtractive cloning strategies. Furthermore, cDNAs found in libraries of certain tissues and not others can be identified using appropriate electronic screening methods.

High throughput genome- or gene-based biology allows new approaches to the identification and cloning of target genes for useful immune responses for the prevention and vaccine therapy of diseases such as cancer and autoimmunity.

In a first aspect, the present invention relates to CASB618 polypeptides. Such peptides include isolated polypeptides comprising an amino acid sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include those comprising the amino acid of SEQ ID NO:2.

Further peptides of the present invention include isolated polypeptides in which the amino acid sequence has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include the polypeptide of SEQ ID NO:2.

Further peptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:1.

The invention also provides an immunogenic fragment of a CASB618 polypeptide, that is a contiguous portion of the CASB618 polypeptide which has the same or similar immunogenic properties to the polypeptide comprising the amino acid seqeunce of SEQ ID NO:2. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the CASB618 polypeptide. Such an immunogenic fragment may include, for example, the CASB618 polypeptide lacking an N-terminal leader sequence, a transmembrane domain or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of CASB618 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet

more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

Peptide fragments incorporating an epitope of CASB618 typically will comprise at least 7, preferably 9 or 10 contiguous amino acids from SEQ ID NO:2. Preferred epitopes are shown in SEQ ID NO:5 to SEQ ID NO:77.

Peptides that incorporate these epitopes form a preferred aspect of the present invention. Mimotopes which have the same characteristics as these epitopes, and immunogens comprising such mimotopes which generate an immune response which cross-react with an epitope in the context of the CASB618 molecule, also form part of the present invention.

The present invention, therefore, includes isolated peptides encompassing these epitopes themselves, and any mimotope thereof. The meaning of mimotope is defined as an entity which is sufficiently similar to the native CASB618 epitope so as to be capable of being recognised by antibodies which recognise the native molecule; (Gheysen, H.M., et al., 1986, Synthetic peptides as antigens. Wiley, Chichester, Ciba foundation symposium 119, p130-149; Gheysen, H.M., 1986, Molecular Immunology, 23,7, 709-715); or are capable of raising antibodies, when coupled to a suitable carrier, which antibodies cross-react with the native molecule.

Peptide mimotopes of the above-identified epitopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides of the present invention may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine to the epitope. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. This reduces the conformational degrees of freedom of the peptide, and thus increases the probability that the peptide is presented in a conformation which most closely resembles that of the peptide as found in the context of the whole molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide. Those skilled in the art will realise that such modified peptides, or

mimotopes, could be a wholly or partly non-peptide mimotope wherein the constituent residues are not necessarily confined to the 20 naturally occurring amino acids. In addition, these may be cyclised by techniques known in the art to constrain the peptide into a conformation that closely resembles its shape when the peptide sequence is in the context of the whole molecule. A preferred method of cyclising a peptide comprises the addition of a pair of cysteine residues to allow the formation of a disulphide bridge.

Further, those skilled in the art will realise that mimotopes or immunogens of the present invention may be larger than the above-identified epitopes, and as such may comprise the sequences disclosed herein. Accordingly, the mimotopes of the present invention may consist of addition of N and/or C terminal extensions of a number of other natural residues at one or both ends. The peptide mimotopes may also be retro sequences of the natural sequences, in that the sequence orientation is reversed; or alternatively the sequences may be entirely or at least in part comprised of D-stereo isomer amino acids (inverso sequences). Also, the peptide sequences may be retro-inverso in character, in that the sequence orientation is reversed and the amino acids are of the D-stereoisomer form. Such retro or retro-inverso peptides have the advantage of being non-self, and as such may overcome problems of self-tolerance in the immune system.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the epitopes of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native peptide. This approach may have significant advantages by allowing the possibility of identifying a peptide with enhanced immunogenic properties, or may overcome any potential self-antigen tolerance problems which may be associated with the use of the native peptide sequence. Additionally this technique allows the identification of a recognition pattern for each native-peptide in terms of its shared chemical properties amongst recognised mimotope sequences.

The covalent coupling of the peptide to the immunogenic carrier can be carried out in a manner well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester, utilising common commercially available heterobifunctional linkers

such as CDAP and SPDP (using manufacturers instructions). After the coupling reaction, the immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc.

The types of carriers used in the immunogens of the present invention will be readily known to the man skilled in the art. The function of the carrier is to provide cytokine help in order to help induce an immune response against the peptide. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins (TT and DT), or recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein derivative of tuberculin (PPD). Alternatively the mimotopes or epitopes may be directly conjugated to liposome carriers, which may additionally comprise immunogens capable of providing T-cell help. Preferably the ratio of mimotopes to carrier is in the order of 1:1 to 20:1, and preferably each carrier should carry between 3-15 peptides.

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

Another preferred method of presenting the peptides of the present invention is in the context of a recombinant fusion molecule. For example, EP 0 421 635 B describes the use of chimaeric hepadnavirus core antigen particles to present foreign peptide sequences in a virus-like particle. As such, immunogens of the present invention may comprise peptides presented in chimaeric particles consisting of hepatitis B core antigen. Additionally, the recombinant fusion proteins may comprise the mimotopes of the present invention and a carrier protein, such as NS1 of the influenza virus. For any recombinantly expressed protein which forms part of the present invention, the nucleic acid which encodes said immunogen also forms an aspect of the present invention.

Peptides used in the present invention can be readily synthesised by solid phase procedures well known in the art. Suitable syntheses may be performed by utilising "T-boc" or "F-moc" procedures. Cyclic peptides can be synthesised by the solid phase

procedure employing the well-known "F-moc" procedure and polyamide resin in the fully automated apparatus. Alternatively, those skilled in the art will know the necessary laboratory procedures to perform the process manually. Techniques and procedures for solid phase synthesis are described in 'Solid Phase Peptide Synthesis: A Practical Approach' by E. Atherton and R.C. Sheppard, published by IRL at Oxford University Press (1989). Alternatively, the peptides may be produced by recombinant methods, including expressing nucleic acid molecules encoding the mimotopes in a bacterial or mammalian cell line, followed by purification of the expressed mimotope. Techniques for recombinant expression of peptides and proteins are known in the art, and are described in Maniatis, T., Fritsch, E.F. and Sambrook et al., *Molecular cloning, a laboratory manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

The polypeptides or immunogenic fragment of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins.

Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenza* B and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another immunological fusion partner is the protein known as LYTA. Preferably the C terminal portion of the molecule is used. Lyta is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LYTA, (coded by the lytA gene {Gene, 43 (1986) page 265-272} an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of E.coli C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the Lyta molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to CASB618 polynucleotides. Such polynucleotides include isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2. In this regard, polypeptides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding the polypeptide of SEQ ID NO:2.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence that has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to SEQ ID NO:1 over the entire length of SEQ ID NO:1. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identiy are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the polynucleotide of SEQ ID NO:1 as well as the polynucleotide of SEQ ID NO:1. Said polynucleotide can be inserted in a suitable plasmid or recombinant microrganism vector and used for

immunization (see for example Wolff et. al., Science 247:1465-1468 (1990); Corr et. al., J. Exp. Med. 184:1555-1560 (1996); Doe et. al., Proc. Natl. Acad. Sci. 93:8578-8583 (1996)). The invention also provides polynucleotides which are complementary to all the above described polynucleotides.

The invention also provides a fragment of a CASB618 polynucleotide which when administered to a subject has the same immunogenic properties as the polynucleotide of SEQ ID NO:1.

The invention also provides a polynucleotide encoding an immunological fragment of a CASB618 polypeptide as hereinbefore defined.

The nucleotide sequence of SEQ ID NO:1 shows homology with Homo sapiens chromosome 15 clone 163_P_10 map 15 (accession GB_HTG4:AC009700) . The nucleotide sequence of SEQ ID NO:1 is a cDNA sequence and comprises a polypeptide encoding sequence (nucleotide 259 to 1219) encoding a polypeptide of 320 amino acids, the polypeptide of SEQ ID NO:2. The nucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 or it may be a sequence other than the one contained in SEQ ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of the SEQ ID NO:2 is not related to any other protein of known function, except to Caenorhabditis elegans hypothetical 42.1 kd protein c06e1.3 (accession P34298) .

Preferred polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides, immunological fragments and polynucleotides of the present invention have at least one activity of either SEQ ID NO:1 or SEQ ID NO:2, as appropriate.

The present invention also relates to partial or other incomplete polynucleotide and polypeptide sequences which were first identified prior to the determination of the corresponding full length sequences of SEQ ID NO:1 and SEQ ID NO:2.

Accordingly, in a further aspect, the present invention provides for an isolated polynucleotide which:

(a) comprises a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;

(b) has a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% identity, to SEQ ID NO:1 over the entire length of SEQ ID NO:3;

(c) the polynucleotide of SEQ ID NO:3.

The nucleotide sequence of SEQ ID NO:3 is derived from EST (Expressed Sequence Tag) sequences. It is recognised by those skilled in the art that there will inevitably be some nucleotide sequence reading errors in EST sequences (see Adams, M.D. *et al*, Nature 377 (supp) 3, 1995). Accordingly, the nucleotide sequence of SEQ ID NO:3 is therefore subject to the same inherent limitations in sequence accuracy.

Polynucleotides of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of human colon cancer, lung cancer, uterine cancer, and fetal tissues (for example Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring harbor Laboratory Press, Cold Spring harbor, N.Y. (1989)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence

for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further embodiments of the present invention include polynucleotides encoding polypeptide variants which comprise the amino acid sequence of SEQ ID NO:2 and in which several, for instance from 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1, amino acid residues are substituted, deleted or added, in any combination.

Polynucleotides which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification (PCR) reaction, to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding paralogs from human sources and orthologs and paralogs from species other than human) that have a high sequence similarity to SEQ ID NO:1. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical, most preferably 95% identical to that of the referent. The probes or primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides. Particularly preferred primers will have between 20 and 25 nucleotides. In particular, polypeptides or polynucleotides derived from sequences from homologous animal origin could be used as immunogens to obtain a cross-reactive immune response to the human gene.

A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process which comprises the steps of

screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C. Thus the present invention also includes polynucleotides obtainable by screening an appropriate library under stingent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof.

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is short at the 5' end of the cDNA.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., PNAS USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the 'missing' 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analysed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a

separate full-length PCR using the new sequence information for the design of the 5' primer.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to an expression system which comprises a polynucleotide of the present invention, to host cells which are genetically engineered with such expression sytems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Preferably the proteins of the invention are coexpressed with thioredoxin in trans (TIT). Coexpression of thioredoxin in trans versus in cis is preferred to keep antigen free of thioredoxin without the need for protease. Thioredoxin coexpression eases the solubilisation of the proteins of the invention. Thioredoxin coexpression has also a significant impact on protein purification yield, on purified-protein solubility and quality.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (supra). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelian Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella , Shigella, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography,

hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

Another important aspect of the invention relates to a method for inducing , re-inforcing or modulating an immunological response in a mammal which comprises inoculating the mammal with a fragment or the entire polypeptide or polynucleotide of the invention, adequate to produce antibody and/or T cell immune response for prophylaxis or for therapeutic treatment of cancer and autoimmune disease and related conditions. Yet another aspect of the invention relates to a method of inducing, re-inforcing or modulating immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector or cell directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce immune responses for prophylaxis or treatment of said mammal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces, re-inforces or modulates an immunological response in that mammal to a polypeptide of the present invention wherein the composition comprises a polypeptide or polynucleotide of the invention or an immunological fragment thereof as herein before defined.The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

A further aspect of the invention relates to the *in vitro* induction of immune responses to a fragment or the entire polypeptide or polynucleotide of the present invention or a molecule comprising the polypeptide or polynucleotide of the present invention, using cells from the immune system of a mammal, and reinfusing these activated immune cells of the mammal for the treatment of disease. Activation of the cells from the immune system is achieved by in vitro incubation with the entire polypeptide or polynucleotide of the present invention or a molecule comprising the polypeptide or polynucleotide of the present invention in the presence or absence of various immunomodulator molecules. A further aspect of the invention relates to the immunization of a mammal by administration of antigen presenting cells modified by *in vitro* loading with part or the entire polypeptide of the present invention or a molecule comprising the polypeptide of the present invention and administered *in vivo* in an immunogenic way. Alternatively, antigen presenting cells can be transfected *in vitro* with a vector containing a fragment or the entire polynucleotide of the present invention or a molecule comprising the polynucleotide of the present invention, such as to express the corresponding polypeptide, and administered *in vivo* in an immunogenic way.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

An immune response may be broadly distinguished into two extreme catagories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type

immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).
3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1;

preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

This invention also relates to the use of polynucleotides, in the form of primers derived from the polynucleotides of the present invention, and of polypeptides, in the form of antibodies or reagents specific for the polypeptide of the present invention, as diagnostic reagents.

The identification of genetic or biochemical markers in blood or tissues that will enable the detection of very early changes along the carcinogenesis pathway will help in determining the best treatment for the patient. Surrogate tumour markers, such as polynucleotide expression, can be used to diagnose different forms and states of cancer. The identification of expression levels of the polynucleotides of the invention will be useful in both the staging of the cancerous disorder and grading the nature of the cancerous tissue. The staging process monitors the advancement of the cancer and is determined on the presence or absence of malignant tissue in the areas biopsied. The polynucleotides of the invention can help to perfect the staging process by identifying markers for the aggresivity of a cancer, for example the presence in different areas of the body. The grading of the cancer describes how closely a tumour resembles normal tissue of its same type and is assessed by its cell morphology and other markers of differentiation. The polynucleotides of the invention can be useful in determining the tumour grade as they can help in the determination of the differentiation status of the cells of a tumour.

The diagnostic assays offer a process for diagnosing or determining a susceptibility to cancers, autoimmune disease and related conditions through diagnosis by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. This method of diagnosis is known as differential expression. The expression of a particular gene is compared between a diseased tissue and a normal tissue. A difference between the polynucleotide-related gene, mRNA, or protein in the two tissues is compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a change in the gene, or a gene which regulates it, in the tissue of the human that was suspected of being diseased.

Decreased or increased expression can be measured at the RNA level. PolyA RNA is first isolated from the two tissues and the detection of mRNA encoded by a gene corresponding to a differentially expressed polynucleotide of the invention can be

detected by, for example, in situ hybridization in tissue sections, reverse trascriptase-PCR, using Northern blots containing poly A+ mRNA, or any other direct or inderect RNA detection method. An increased or decreased expression of a given RNA in a diseased tissue compared to a normal tissue suggests that the transcript and/or the expressed protein has a role in the disease. Thus detection of a higher or lower level of mRNA corresponding to SEQ ID NO 1 or 3 relative to normal level is indicative of the presence of cancer in the patient.

mRNA expression levels in a sample can be determined by generation of a library of expressed sequence tags (ESTs) from the sample. The relative representation of ESTs in the library can be used to assess the relative representation of the gene transcript in the starting sample. The EST analysis of the test can then be compared to the EST analysis of a reference sample to determine the relative expression levels of the polynucleotide of interest.

Other mRNA analyses can be carried out using serial analysis of gene expression (SAGE) methodology (Velculescu et. Al. Science (1995) 270:484) , differential display methodology (For example, US 5,776,683) or hybridization analysis which relies on the specificity of nucleotide interactions.

Alternatively, the comparison could be made at the protein level. The protein sizes in the two tissues may be compared using antibodies to detect polypeptides in Western blots of protein extracts from the two tissues. Expression levels and subcellular localization may also be detected immunologically using antibodies to the corresponding protein. Further assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. A raised or decreased level of polypeptide expression in the diseased tissue compared with the same protein expression level in the normal tissue indicates that the expressed protein may be involved in the disease.

In the assays of the present invention, the diagnosis can be determined by detection of gene product expression levels encoded by at least one sequence set forth in SEQ ID NOS: 1 or 3.

A comparison of the mRNA or protein levels in a diseased versus normal tissue may also be used to follow the progression or remission of a disease.

A large number of polynucleotide sequences in a sample can be assayed using polynucleotide arrays. These can be used to examine differential expression of genes and to determine gene function. For example, arrays of the polynucleotide sequences SEQ ID NO: 1 or 3 can be used to determine if any of the polynucleotides are differentially expressed between a normal and cancer cell. In one embodiment of the invention, an array of oligonucleotides probes comprising the SEQ ID NO: 1 or 3 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

"Diagnosis" as used herein includes determination of a subject's susceptibility to a disease, determination as to whether a subject presently has the disease, and also the prognosis of a subject affected by the disease.

The present invention, further relates to a diagnostic kit for performing a diagnostic assay which comprises:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1 or 3, or a fragment thereof ;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2.

The nucleotide sequences of the present invention are also valuable for chromosomal localisation. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating

those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined.

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

In a further aspect the invention provides an antibody immunospecific for a polypeptide according to the invention or an immunological fragment thereof as hereinbefore defined. Preferably the antibody is a monoclonal antibody

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.
The antibody of the invention may also be employed to prevent or treat cancer, particularly ovarian and colon cancer, autoimmune disease and related conditions.

Another aspect of the invention relates to a method for inducing or modulating an immunological response in a mammal which comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect or ameliorate the symptoms or progression of the disease. Yet another aspect of the invention relates to a method of inducing or modulating immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

It will be appreciated that the present invention therefore provides a method of treating abnormal conditions such as, for instance, cancer and autoimmune diseases, in particular, ovarian and colon cancer, related to either a presence of, an excess of, or an under-expression of, CASB618 polypeptide activity.

The present invention further provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the CASB618 polypeptide. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)). Screening methods will be known to those skilled in the art. Further screening methods may be found in for example D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995) and references therein.

Thus the invention provides a method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of the invention which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presense of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

The polypeptide of the invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. Well known screening methods may also be used to identify agonists and antagonists of the polypeptide of the invention which compete with the binding of the polypeptide of the invention to its receptors, if any.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such polypeptides, which comprises:

(a) a polypeptide of the present invention;

(b) a recombinant cell expressing a polypeptide of the present invention;

(c) a cell membrane expressing a polypeptide of the present invention; or

(d) antibody to a polypeptide of the present invention;
which polypeptide is preferably that of SEQ ID NO:2.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide, by:

(a) determining in the first instance the three-dimensional structure of the polypeptide;

(b) deducing the three-dimensional structure for the likely reactive or binding site(s) of an agonist, antagonist or inhibitor;

(c) synthesing candidate compounds that are predicted to bind to or react with the deduced binding or reactive site; and

(d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

Gene therapy may also be employed to effect the endogenous production of CASB618 polypeptide by the relevant cells in the subject. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000μg of protein, preferably 2-100μg, most preferably 4-40μg. An optimal amount for a particular vaccine can be

ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research *12(1):* 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., J. Molec. Biol. *215:* 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al.*, J. Mol. Biol. *215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

The preferred algorithm used is FASTA. The preferred parameters for polypeptide or polynuleotide sequence comparison using this algorithm include the following:
Gap Penalty:12
Gap extension penalty: 4
Word size: 2, max 6

Preferred parameters for polypeptide sequence comparison with other methods include the following:
1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%,etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity(divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quntified by determining the degree of identity and/or similarity between the sequences being compared as hereinbefore described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional

equivalent of a polynucleotide or polypeptide in another species and "paralog" meaning a functionally similar sequence when considered within the same species.

FIGURE LEGENDS

Figure 1

Figure 1 shows expression levels of CASB618 in matched colon normal and tumoral samples. The values are given in equivalent actin level.

N refers to normal colon, T refers to colon tumor.

Figure 2

Figure 2A, 2B, 2C and 2D show the real-time PCR data of CASB618 expression in normal tissues.

The abbreviations stand for:

Figure 2A: Co: colon; Ce: cervix; Bra: brain: Bo_Ma: bone marrow; Bl: bladder; Ao: aorta; Ad_Gl: adrenal gland

Figure 2B: Ov: ovary; Oe: Oesophage; Ly_No: lymph node; Lu: lung; Li: liver; Ki: kidney; Il: Ileum; He: heart; Fa_Tu: fallopian tube

Figure 2C: Sp: spleen; Sm_In: small intestine; Sk_Mu: skeletal muscle; Sk: skin; Re: rectum; Pr: prostate; Pl: placenta; Pa_Thy: parathyroid gland

Figure2D: Tr: trachea; Thy: thyroid; Te: testis; St: stomach; Spl: spleen

Figure 3

Figure 3 shows a SDS-PAGE gel (12,5 %) of the *E. coli* AR120/pRIT15081 extract, with or without induction with nalidixic acid; revealed with monoclonal antibody anti-NS1. Lane 1 is the Molecular weight marker; lane 2 shows the *E. coli* AR120/pRIT15081 3hrs no induction; lane 3 shows the *E. coli* AR120/pRIT15081 3hrs induced; lane 4 shows the *E. coli* AR120/pRIT15081 4h30 no induction; lane 5 shows the *E. coli* AR120/pRIT15081 4h30 induced.

Figure 4

Figure 4 shows SDS-PAGE gels of purified CASB618.

Lanes 1 and 8 show the Molecular weight marker; lane 2 shows the lysed cell pellet; lane 3 shows the 3 purified protein before dialyse; lane 4 shows the purified dialysed protein; lane 6 shows the purified dialysed protein 0.22µm.

EXAMPLES

Example 1

Real-time RT-PCR analysis

Real-time RT-PCR (U. Gibson. 1996. Genome Research: 6,996) is used to compare mRNA transcript abundance of the candidate antigen in matched tumour and normal colon tissues from multiple patients. In addition, mRNA levels of the candidate gene in a panel of normal tissues are evaluated by this approach.

Total RNA from normal and tumour colon is extracted from snap frozen biopsies using TriPure reagent (Boehringer). Total RNA from normal tissues is purchased from InVitrogen or is extracted from snap frozen biopsies using TriPure reagent. Poly-A^+ mRNA is purified from total RNA after DNAase treatment using oligo-dT magnetic beads (Dynal). Quantification of the mRNA is performed by spectrofluorimetry (VersaFluor, BioRad) using SybrII dye (Molecular Probes). Primers for real-time PCR amplification are designed with the Perkin-Elmer Primer Express software using default options for TaqMan amplification conditions.

Real-time reactions are assembled according to standard PCR protocols using 2 ng of purified mRNA for each reaction. SybrI dye (Molecular Probes) is added at a final dilution of 1/75000 for real-time detection. Amplification (40 cycles) and real-time detection is performed in a Perkin-Elmer Biosystems PE7700 system using conventional instrument settings. Ct values are calculated using the PE7700 Sequence Detector software. Two Ct values are obtained for each patient sample: the tumour Ct (CtT) and the matched normal colon Ct (CtN). Ct values obtained by real-time PCR are log-linearly related to the copy number of the target template. As the efficiency of PCR amplification under the prevailing experimental conditions is close to the theoretical amplification efficiency, $2^{(CtN-CtT)}$ is an estimate of the relative transcript levels in the two tissues (i.e. fold mRNA over-expression in tumor). Real-time PCR reactions are performed on biopsies from 23 patients. Some patient samples were measured twice. The level of mRNA over-expression is calculated as described for each patient. average level of mRNA over-expression for the candidate antigen and the proportion of patients over-expressing the candidate antigen is then calculated from this data set. The individual values are standardised with respect to actin in the same sample (ratio) and are shown in

figure 1. A value of 1 thus corresponds to the same level of actin expression. The results are shown in a logarithmic scale.

A total of 81 normal tissue samples, representing 28 different tissues, were also tested by the same procedure. Ct values for the candidate antigen were compared to those of actin obtained with the same tissue sample. Standardised values are shown in figures 2A-D.

Real-time PCR results in colon cancer/normal colon sample

Summary

Patients over-expressing CASB618 in colon tumours (%)	Average level of over-expression in colon tumours (fold)
18/23 (78%)	125

Conclusion: CASB618 is overexpressed in a high proportion of tumors with respect to the normal adjacent colon. It is only marginally expressed by other normal tissues, in particular one prostate sample.

Example 2.

DNA microarrays

DNA micro-arrays are used to examine mRNA expression profiles of large collections of genes in multiple samples. This information is used to complement the data obtained by real-time PCR and provides an independent measure of gene expression levels in tumors and normal tissues.

Examples of current technologies for production of DNA micro-arrays include 1) The Affymetrix "GeneChip" arrays in which oligonucleotides are synthetized on the surface of the chip by solid phase chemical synthesis using a photolithographic process 2) DNA spotting technology in which small volumes of a DNA solution are robotically deposited and then immobilized onto the surface of a solid phase (e.g. glass). In both instances, the chips are hybridized with cDNA or cRNA which has been extracted from the tissue of interest (e.g. normal tissue, tumour etc...) and labeled with radioactivity or with a fluorescent reporter molecule. The labeled material is hybridized to the chip and the amount of probe bound to each sequence on the chip is determined using a specialized

scanner. The experiment can be set-up with a single fluorescent reporter (or radioactivity) or, alternatively, can be performed using two fluorescent reporters. In this latter case, each of the two samples is labeled with one of the reporter molecules. The two labeled samples are then hybridized competitively to the sequences on the DNA chip. The ratio of the two fluorescent signals is determined for each sequence on the chip. This ratio is used to calculate the relative abundance of the transcript in the two samples. Detailed protocols are available from a number of sources including "DNA Microarrays: A practical approach. Schena M. Oxford University Press 1999" and the World Wide Web (http://cmgm.stanford.edu/pbrown/protocols/index.html), http://arrayit.com/DNA-Microarray-Protocols/) and specialized distributors (e.g. Affymetrix).

Example 3.

EST profiles

A complementary approach to experimental antigen tissue expression characterization is to explore the human "Expressed Sequence Tags" (ESTs) database. ESTs are small fragments of cDNA made from a collection of mRNA extracted from a particular tissue or cell line. Such databases currently provide a massive amount of ESTs (10^6) from several hundreds of cDNA tissue libraries, including tumoral tissues from various types and states of disease. By means of informatics tools (Blast), a comparison search of the CASB616 sequence is performed in order to have further insight into tissue expression.

EST distribution of CASB618

DbEST accession	ATG lib ID	Description	Category
NCBI:1113567	882	NCI_CGAP_Co3	TC
NCBI:1224225	937	NCI_CGAP_Co2	TC
NCBI:1271870	988	NCI_CGAP_Co12	TC
NCBI:1316079	889	NCI_CGAP_Thy1	TA
NCBI:2035497	1079	NCI_CGAP_Co8	TC
NCBI:2048268	1079	NCI_CGAP_Co8	TC
NCBI:2054603	1079	NCI_CGAP_Co8	TC
NCBI:2081390	1079	NCI_CGAP_Co8	TC
NCBI:2129969	1079	NCI_CGAP_Co8	TC
NCBI:2489139	1728	Soares_Dieckgraefe_	Dc
NCBI:2489206	1728	Soares_Dieckgraefe_	Dc
NCBI:2600163	882	NCI_CGAP_Co3	TC
NCBI:2641414	882	NCI_CGAP_Co3	TC

NCBI:2831741	937 NCI_CGAP_Co2	TC
NCBI:2914582	910 NCI_CGAP_Pr22	NP
NCBI:3111692	1728 Soares_Dieckgraefe_	Dc
NCBI:3112040	1728 Soares_Dieckgraefe_	Dc
NCBI:3043263	1460 NCI_CGAP_Pan1	Tep
NCBI:3119272	1728 Soares_Dieckgraefe_	Dc
NCBI:3138950	882 NCI_CGAP_Co3	TC
NCBI:3181303	1728 Soares_Dieckgraefe_	Dc
NCBI:2908798	882 NCI_CGAP_Co3	TC
NCBI:2909226	937 NCI_CGAP_Co2	TC

TC: colon tumor; Dc: diseased colon; NP; normal prostate; Tep: epithelial tumor; Ta: other tumor type.

The high proportion of colon cancer ESTs thus clearly suggests an overexpression of this gene in colon cancer. Additionally, other tumors (pancreas, thyroid) could also express the gene.

Example 4

Northern-Southern blot analysis

Limited amounts of mixed tumour and matched normal colon cDNA are amplified by Advantage PCR (see above). Messenger RNA from multiple normal tissues is also amplified using the same procedure. The amplified cDNA (1 µg) is electrophoresed on a 1.2% agarose gel and transferred onto a nylon membrane. The membrane is hybridised (AlkPhos Direct System) with a probe prepared using a fragment of the candidate TAA cDNA. Northern-Southern analysis provides information on transcript size, presence of splice variants and transcript abundance in tumour and normal tissues.

Example 5

Northern Blot Analysis

Northern blots are produced according to standard protocols using 1 µg of poly A+ mRNA. Radioactive probes are prepared using the Ready-to-Go system (Pharmacia).

Example 6

Identification of the full length cDNA sequence

Colon tumour cDNA libraries are constructed using the Lambda Zap II system (Stratagene) from 5 µg of polyA+ mRNA. The supplied protocol is followed except that SuperscriptII (Life Technologies) is used for the reverse transcription step. Oligo dT-primed and random-primed libraries are constructed. About 1.5 x106 independent phages are plated for each screening of the library. Phage plaques are transferred onto nylon filters and hybridised using a cDNA probe labelled with AlkPhos Direct. Positive phages are detected by chemiluminescence. Positive phage are excised from the agar plat, eluted in 500µl SM buffer and confirmed by gene-specific PCR. Eluted phages are converted to single strand M13 bacteriophage by in vivo excision. The bacteriophage is then converted to double strand plasmid DNA by infection of E. coli. Infected bacteria are plated and submitted to a second round of screening with the cDNA probe. Plasmid DNA is purified from positive bacterial clones and sequenced on both strands.

When the full length gene cannot be obtained directly from the cDNA library, missing sequence is isolated using RACE technology (Marathon Kit, ClonTech.). This approach relies on reverse transcribing mRNA into double strand cDNA, ligating linkers onto the ends of the cDNA and amplifying the desired extremity of the cDNA using a gene-specific primer and one of the linker oligonucleotides. Marathon PCR products are cloned into a plasmid (pCRII-TOPO, InVitrogen) and sequenced.

The obtained sequence (SEQ ID NO:1) has a putative open reading frame of 259 amino acids (SEQ ID NO:2). The deduced protein sequence was submitted to prediction algorithms for cellular localisation (PSORT: http://psort.nibb.ac.jp/ and TopPred: http://www.biokemi.su.se/~server/toppred2/toppred_source.html). It is predicted to have 4 to 5 transmembrane segments; only one of the 2 methods used predicts the signal sequence. There is a potential leucine zipper motif overlapping one of the predicted transmembrane segments. There are 3 potential N-glycosylation sites. Subcellular localisation is unclear, plasma membrane being the most probable.

Example 7 :

7.1 Expression and purification of tumour-specific antigens

Expression in microbial hosts, or alternatively *in vitro* transcription/translation, is used to

produce the antigen of the invention for vaccine purposes and to produce protein fragments or whole protein for rapid purification and generation of antibodies needed for characterization of the naturally expressed protein by immunohistochemistry or for follow-up of purification.

Recombinant proteins may be expressed in two microbial hosts, *E. coli* and in yeast (such as Saccharomyces cerevisiae or Pichia pastoris).Pichia. This allows the selection of the expression system with the best features for this particular antigen production. In general, the recombinant antigen will be expressed in *E. coli* and the reagent protein expressed in yeast.

The expression strategy first involves the design of the primary structure of the recombinant antigen. In general an expression fusion partner (EFP) is placed at the N terminal extremity to improve levels of expression that could also include a region useful for modulating the immunogenic properties of the antigen, an immune fusion partner (IFP). In addition, an affinity fusion partner (AFP) useful for facilitating further purification is included at the C-terminal end.

When the recombinant strains are available, the recombinant product is characterized by the evaluation of the level of expression and the prediction of further solubility of the protein by analysis of the behavior in the crude extract.

After growth on appropriate culture medium and induction of the recombinant protein expression, total extracts are analyzed by SDS-PAGE. The recombinant proteins are visualized in stained gels and identified by Western blot analysis using specific antibodies.

A comparative evaluation of the different versions of the expressed antigen will allow the selection of the most promising candidate that is to be used for further purification and immunological evaluation.

Expression in *E. coli* AR120

The following construct was designed and made: the gene CASB618 carrying deletions of the N-terminus and C-terminus (Δ 1-74; Δ 247-320 aa) was cloned in vector pMG81(pr PL long), with the addition of an IFP (NS1 DNA sequence encoding the N-terminal 1 to 81 amino acids of the NS1 protein of Influenza virus) at the N-terminus, and a C-terminal histidine tail (SEQ ID NO:4)

NS1- Met Thr Met | C 61-8 (75 – 246) | Thr Ser Gly 6xHIS

The obtained plasmid is called pRIT 15081. The acid nalidixic inductible host cell *E. coli* AR120 is used. Induction of three litres of *E.coli* cultures in LB medium + Kannamycin was obtained by adding acid nalidixic to a final concentration of 60ng/ml . The cultures were incubated 4h30 at 37°C .

The pellet obtained after centrifugation of the induced cultures was resuspended in 60 ml of PBS buffer. The cells were subsequently lysed using a French press. The lysate was then centrifugation for 20 minutes at 16000g. We found the expressed protein in the pellet (figure 3).

The purification scheme follows a classical approach based on the presence of an His affinity tail in the recombinant protein. In a typical experiment the disrupted cells are filtered and the acellular extracts loaded onto an Ion Metal Affinity Chromatography (IMAC; Ni^{++}NTA from Qiagen) that will specifically retain the recombinant protein. The retained proteins are eluted by 0-500 mM Imidazole gradient (possibly in presence of a detergent) in a phosphate buffer.

The purification scheme is detailed below.

Solubilization of the pellet in GuHCl 6M

⇩

IMAC: Qiagen NTA NI++

Equilibration buffer : NaH2Po4 100mM PH 8
Tris 10mM
NaCl 150mM
GuHCl 6 M

Sample : pellet in GuHCl 6M

Wash buffer :	NaH2PO4	100mM	PH 6.3
	Tris	10mM	
	urea	8 M	
Elution buffer :	NaH2PO4	100mM	PH 4,5
	Tris	10mM	
	Imidazole	500mM	
	urea	8 M	

⇩

Eluted protein in 500mM imidazole + 8M urea

⇩

DIALYSIS

- PBS PH 7.5 + sarkosyl 2% + 6M urea 1hrs
- " 4M urea 1hrs
- " 2M urea 1hrs
- " 0M urea overnight 4°C

⇩

Freeze, filtration 0,22µm

An estimation of the final concentration (1 mg / ml) is obtained by a Lowry protein assay on the final purified product (see figure 4).

In vitro transcription/translation

The CASB618 gene product was characterised by coupled transcription / translation in vitro. Full-length coding sequence of clone CASB618 was cloned into SP72 vector (Promega), a vector allowing in vitro transcription. In vitro expression using TNT T7 coupled reticulocyte lysate (Promega cat.n° L4611) with incorporation of S35 methionine shows a product of 35Kd, which is reduced to 30 Kd in presence of canine pancreatic microsomal membranes (Promega cat. N° Y4041). This result suggests processing of the signal peptide in accordance with the signal peptide prediction of 47 amino acids (, and suggests that the protein in vivo is membrane anchored or secreted. For these experiments, protocols recommended by Promega were followed.

7.2 Antibody production and immunohistochemistry

Small amounts of relatively purified protein can be used to generate immunological tools in order to

a) detect the expression by immunohistochemistry in normal or cancer tissue sections;

b) detect the expression, and to follow the protein during the purification process (ELISA/ Western Blot); or

c) characterise/ quantify the purified protein (ELISA).

7.2.1 Polyclonal antibodies:

Immunization

2- 3 Rabbits are immunised , intramuscularly (I.M.) , 3 times at 3 weeks intervals with 100µg of protein, formulated in the adjuvant 3D-MPL/QS21. Three weeks after each immunisation a blood sample is taken and the antibody titer estimated in the serum by ELISA using the protein as coating antigen following a standard protocol.

ELISA

96 well microplates (maxisorb Nunc) are coated with 5µg of protein overnight at 4°C. After 1hour saturation at 37°C with PBS NCS 1%, serial dilution of the rabbit sera is added for 1H 30 at 37°C (starting at 1/10). After 3 washings in PBS Tween, anti rabbit biotinylated anti serum (Amersham) is added (1/5000). Plates are washed and peroxydase coupled streptavidin (1/5000) is added for 30 min at 37°C. After washing, 50µl TMB (BioRad) is added for 7 min and the reaction then stopped with H_2SO_4 0.2M. The OD can be measured at 450 nm and midpoint dilutions calculated by SoftmaxPro.

7.2.2 Monoclonal antibodies:

Immunization

5 BALB/c mice are immunized 3 times at 3 week intervals with 5 µg of purified protein. Bleedings are performed 14 days post II and 1 week post 3. The sera are tested by Elisa on purified protein used as coated antigen. Based on these results (midpoint dilution > 10000) one mouse is selected for fusion

Fusion/ HATselection

Spleen cells are fused with the SP2/0 myeloma according to a standard protocol using PEG 40% and DMSO 5%. Cells are then seeded in 96 well plates 2.5×10^4 - 10^5

cells/well and resistant clones will be selected in HAT medium. The supernatant of these hybridomas will be tested for their content in specific antibodies and when positive, will be submitted to 2 cycles of limited dilution . After 2 rounds of screening, 3 hybridomas will be chosen for ascitis production.

7.2.3 Immunohistochemistry

When antibodies are available, immuno staining is performed on normal or cancer tissue sections, in order to determine :

- the level of expression of the antigen of the invention in cancer relative to normal tissue or
- the proportion of cancer of a certain type expressing the antigen
- if other cancer types also express the antigen
- the proportion of cells expressing the antigen in a cancer tissue

Tissue sample preparation

After dissection, the tissue sample is mounted on a cork disk in OCT compound and rapidly frozen in isopentane previously super cooled in liquid nitrogen (-160°C). The block will then be conserved at -70°C until use. 7-10µm sections will be realised in a cryostat chamber (-20, -30°C).

Staining

Tissue sections are dried for 5 min at room Temperature (RT), fixed in acetone for 10min at RT, dried again, and saturated with PBS 0.5% BSA 5% serum. After 30 min at RT either a direct or indirect staining is performed using antigen specific antibodies. A direct staining leads to a better specificity but a less intense staining whilst an indirect staining leads to a more intense but less specific staining.

7.3 Analysis of human cellular immune responses to the antigen of the invention

The immunological relevance of the antigen of the invention can be assessed by *in vitro* priming of human T cells. All T cell lymphocyte lines and dendritic cells are derived from PBMCs (peripheral blood mononuclear cells) of healthy donors (preferred HLA-A2 subtype). An HLA-A2.1/K^b transgenic mice is also used for screening of HLA-A2.1 peptides.

Newly discovered antigen-specific CD8+ T cell lines are raised and maintained by weekly in vitro stimulation. The lytic activity and the γ-IFN production of the CD8 lines in response to the antigen or antigen derived-peptides is tested using standard procedures.

Two strategies to raise the CD8+ T cell lines are used: a peptide-based approach and a whole gene-based approach. Both approaches require the full-length cDNA of the newly discovered antigen in the correct reading frame to be either cloned in an appropriate delivery system or to be used to predict the sequence of HLA binding peptides.

Peptide-based approach

The HLA-A2 binding peptide sequences are predicted either by the Parker's algorithm (Parker, K. C., M. A. Bednarek, and J. E. Coligan. 1994. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J. Immunol. 152:163 and http://bimas.dcrt.nih.gov/molbio/hla_bind/) or the Rammensee method (Rammensee, Friede, Stevanovic, MHC ligands and peptide motifs: 1st listing, Immunogenetics 41, 178-228, 1995 ; Rammensee, Bachmann, Stevanovic: MHC ligands and peptide motifs. Landes Bioscience 1997, and http://134.2.96.221/scripts/hlaserver.dll/home.htm). Peptides are then screened in the HLA-A2.1/K^b transgenic mice model (Vitiello et al.). The sequence used to perform the prediction is EPHB2v, as it is identical to EPHB2 with an additional C-terminal sequence extension.

a) Predicted epitopes binding the HLA_A0201 allele :

a.1) HLA-A*0201 nonamers

Position	1	2	3	4	5	6	7	8	9	Rammen see score	Parker score°	**SEQ ID NO**
262	F	L	G	G	A	V	V	S	L	31	226.014	**5**
24	L	L	I	V	I	L	V	F	L	30	459.398	**6**
253	T	L	A	T	G	V	L	C	L	29		**7**
203	P	L	Y	G	G	L	A	L	L	28		**8**
149	G	L	P	D	P	V	L	Y	L	28	1107.961	**9**
100	G	L	L	V	G	L	E	G	I	28		**10**
53	W	L	V	R	V	L	L	S	L	27	226.014	**11**

62	F I G A E I V A V	26	101.181	12
260	C L F L G G A V V	25	105.510	13
196	V L L S T P A P L	25	134.369	14
60	S L F I G A E I V	25		15
210	L L T T G A F A L	24	210.633	16
104	G L E G I N I T L	24		17
34	L A A S F L L I L	24		18
222	F A L A S I S S V	23		19
216	F A L F G V F A L	23		20
192	L L S N V L L S T	23		21
138	Y A A E Y A N A L	23		22
33	A L A A S F L L I	23		23
31	F L A L A A S F L	23	540.469	24
21	S V P L L I V I L	23		25
174	H L A G H Y A S A	22		26
112	L T G T P V H Q L	22		27
97	A R V G L L V G L	22		28
91	S A A R V T A R V	22		29
73	S A E W F V G T V	22		30
27	V I L V F L A L A	22		31
308	A A L P D L K C I	21		32
299	I L G D P L H K Q	21		33
258	V L C L F L G G A	21		34
217	A L F G V F A L A	21		35
207	G L A L L T T G A	21		36
40	L I L P G I R G H	21		37
16	H A A G F S V P L	21		38
312	D L K C I T T N L	20		39
234	P L R L G S S A L	20		40
209	A L L T T G A F A	20	101.099	41
26	I V I L V F L A L	20		42
17	A A G F S V P L L	20		43
154	V L Y L A E K F T		222.964	44
244	T Q Y G A A F W W		719.848	45
185	W V A F C F W L L		122.527	46

° Estimate of half time of disassociation of a molecule containing this subsequence

a.2) HLA A02_01 decamers

Position	sequence	Rammensee score*	Parker score°	SEQ ID NO:
33	ALAASFLLIL	29		47
223	ALASISSVPL	26		48
111	TLTGTPVHQL	26		49
209	ALLTTGAFAL	25	458.437	50
23	PLLIVILVFL	25		51

272	YVRPSALRTL	24		52
261	LFLGGAVVSL	24		53
226	SISSVPLCPL	24		54
58	LLSLFIGAEI	24		55
191	WLLSNVLLST	23	291.716	56
61	LFIGAEIVAV	23		57
31	FLALAASFLL	23	569.949	58
16	HAAGFSVPLL	23		59
269	SLQYVRPSAL	22		60
258	VLCLFLGGAV	22		61
252	VTLATGVLCL	22		62
101	LLVGLEGINI	22		63
25	LIVILVFLAL	22		64
24	LLIVILVFLA	22	112.664	65
298	LILGDPLHKQ	21		66
183	TLWVAFCFWL	21	21493.266	67
149	GLPDPVLYLA	21		68
145	ALEKGLPDPV	21		69
96	TARVGLLVGL	21		70
72	FSAEWFVGTV	21		71
175	LAGHYASATL	20		72
148	KGLPDPVLYL	20		73
104	GLEGINITLT	20		74
52	FWLVRVLLSL	20		75
21	SVPLLIVILV	20		76
69	AVHFSAEWFV		251.039	77

° Estimate of half time of disassociation of a molecule containing this subsequence

Briefly, transgenic mice are immunized with adjuvanted HLA-A2 peptides, those unable to induce a CD8 response (as defined by an efficient lysis of peptide-pulsed autologous spleen cells) will be further analyzed in the human system.

Human dendritic cells (cultured according to Romani et al.) will be pulsed with peptides and used to stimulate CD8-sorted T cells (by Facs). After several weekly stimulations, the CD8 lines will be first tested on peptide-pulsed autologous BLCL (EBV-B transformed cell lines). To verify the proper *in vivo* processing of the peptide, the CD8 lines will be tested on cDNA-transfected tumour cells (HLA-A2 transfected LnCaP, Skov3 or CAMA tumour cells).

Whole gene-based approach

CD8+ T cell lines will be primed and stimulated with either gene-gun transfected dendritic cells, retrovirally transduced B7.1-transfected fibroblasts, recombinant pox virus (Kim et al.) or adenovirus (Butterfield et al.) infected dendritic cells. Virus infected cells are very efficient to present antigenic peptides since the antigen is expressed at high level but can only be used once to avoid the over-growth of viral T cells lines.

After alternated stimulations, the CD8+ lines are tested on cDNA-transfected tumour cells as indicated above. Peptide specificity and identity is determined to confirm the immunological validation.

References

Vitiello et al. (L. Sherman), J. Exp. Med., J. Exp. Med, 1991, 173:1007-1015.

Romani et al., J. Exp. Med., 1994, 180:83-93.

Kim et al., J. Immunother., 1997, 20:276-286.

Butterfield et al., J. Immunol., 1998, 161:5607-5613.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

of the cDNA and amplifying the desired extremity of the cDNA using a gene-specific primer and one of the linker oligonucleotides. Marathon PCR products are cloned into a plasmid and sequenced.

Claims

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to SEQ ID NO:2.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence of SEQ ID NO:2.

4. The isolated polypeptide of SEQ ID NO:2.

5. A polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of the immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:2

6. A polypeptide as claimed in any of claims 1 to 5 wherein said polypeptide is part of a larger fusion protein.

7. A polypeptide as claimed in any of claims 1 to 6 chemically conjugated to a carrier protein.

8. An isolated polynucleotide encoding a polypeptide as claimed in any of claims 1 to 6.

9. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

10. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

11. An isolated polynucleotide which comprises a nucleotide sequence which has at least 70% identity to that of SEQ ID NO:1 over the entire length of SEQ ID NO:1; or a nucleotide sequence complementary to said isolated polynucleotide.

12. The isolated polynucleotide as defined in any one of claims 8 to 11 in which the identity is at least 95%.

13. An isolated polynucleotide selected from:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(b) the polynucleotide of SEQ ID NO:1; and

(c) a polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof said polynucleotide encoding a protein which has similar immunogenic properties to those of the protein of sequence ID NO:2

or a nucleotide sequence complementary to said isolated polynucleotide

14. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 8 - 13.

15. A host cell comprising the expression vector of claim 14 or the isolated polynucleotide of claims 8 to 13.

16. A process for producing a polypeptide of claims 1 to 7 comprising culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A vaccine comprising an effective amount of the polypeptide of any one of claims 1 to 7 and a pharmaceutically acceptable carrier.

18. A vaccine comprising an effective amount of the polynucleotide of any one of claims 8 to 13 and a pharmaceutically effective carrier.

19. A vaccine comprising an effective amount of antigen presenting cells, modified by in vitro loading with a polypeptide of any one of claims 1 to 7, or genetically modified in vitro to express a polypeptide of claims 1 to 7 and a pharmaceutically effective carrier.

20. A vaccine as claimed in any one of claims 17 to 19 which additionally comprises a TH-1 inducing adjuvant.

21. A vaccine as claimed in claim 20 in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

22. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

23. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of any one of claims 1 to 5 which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the said polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any one of claims 1 to 7, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

24. A method for the treatment of a subject by immunoprophylaxis or therapy comprising *in vitro* induction of immune responses to a molecule of any one of claims 1 to 5, using *in vitro* incubation of the polypeptide of any one of claims 1 to 7 or the polynucleotide of any one of claims 8 to 13 with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for the treatment of disease.

25. A method as claimed in claim 24 wherein the treatment is for ovarian or colon cancer.

26. An agonist or antagonist to the polypeptide of claims 1 to 5.

27. A compound which is:
(a) an agonist or antagonist to the polypeptide of claims 1 to 5;
(b) isolated polynucleotide of claims 8 to 13; or
(c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of any one of claims 1 to 5;
for use in therapy.

28. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

29. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

30. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

31. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

32. An isolated polynucleotide selected from the group consisting of:
(a) an isolated polynucleotide comprising a nucleotide sequence which has at least 70% identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;
(b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3;
(c) the polynucleotide of SEQ ID NO:3.

33. A live vaccine composition comprising an expression vector or recombinant live micro-organism according to claim 14.

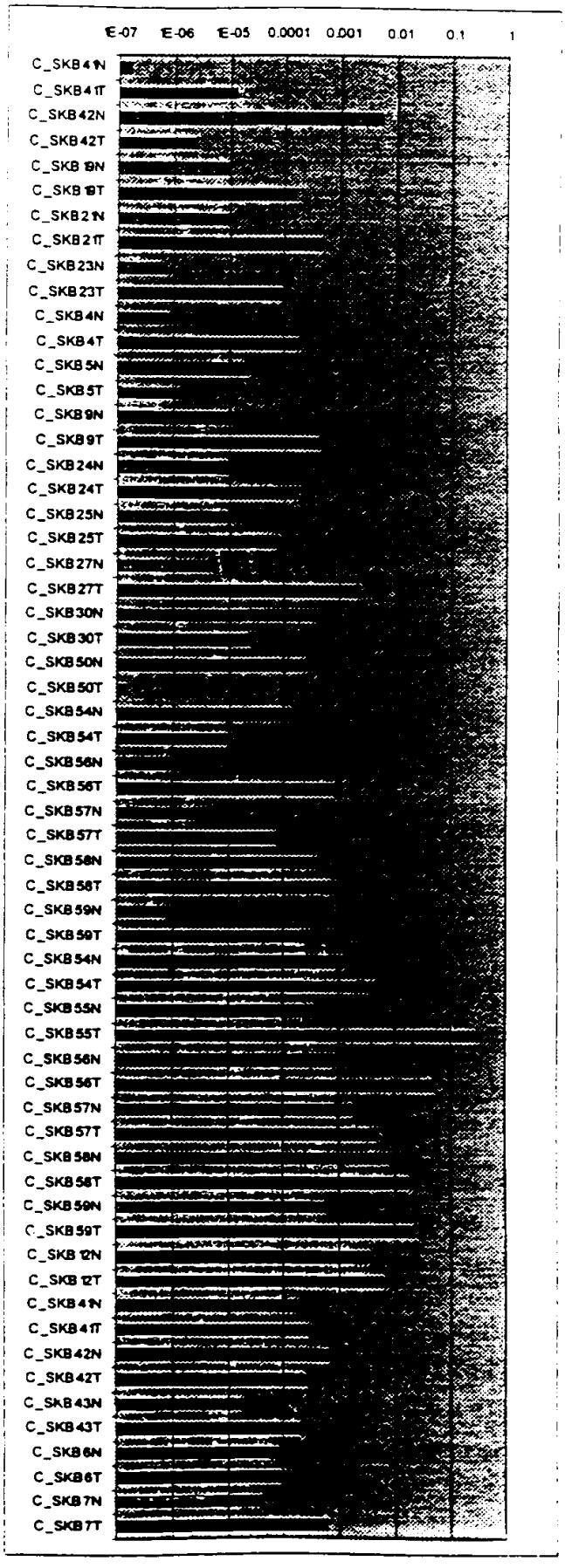


Figure 1: Expression levels of CASB618 in matched colon normal and tumoral samples.

Figure 2A: Expression of CASB618 in normal tissues: Real-time PCR results

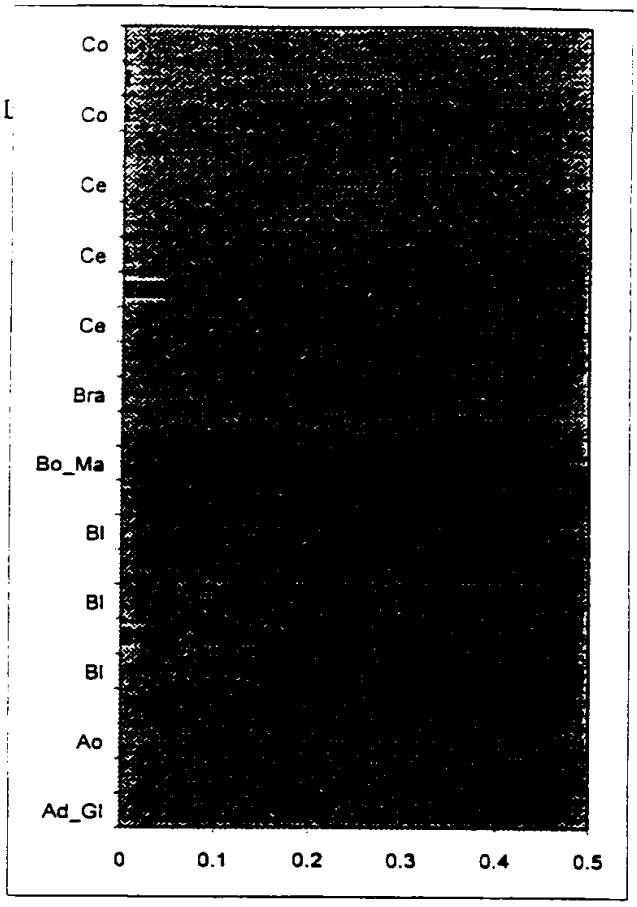


Figure 2B: Real-time PCR data of CASB618 expression in normal tissues (continued)

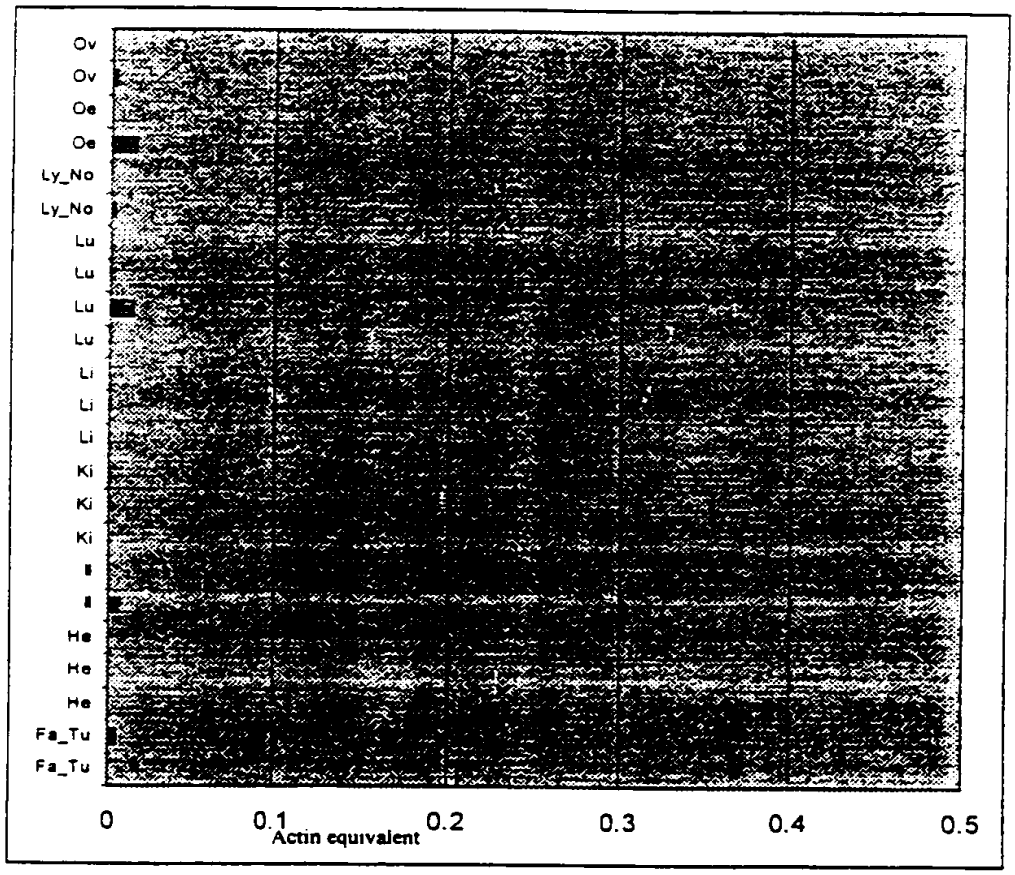


Figure 2C: Real-time PCR data of CASB618 expression in normal tissues (continued)

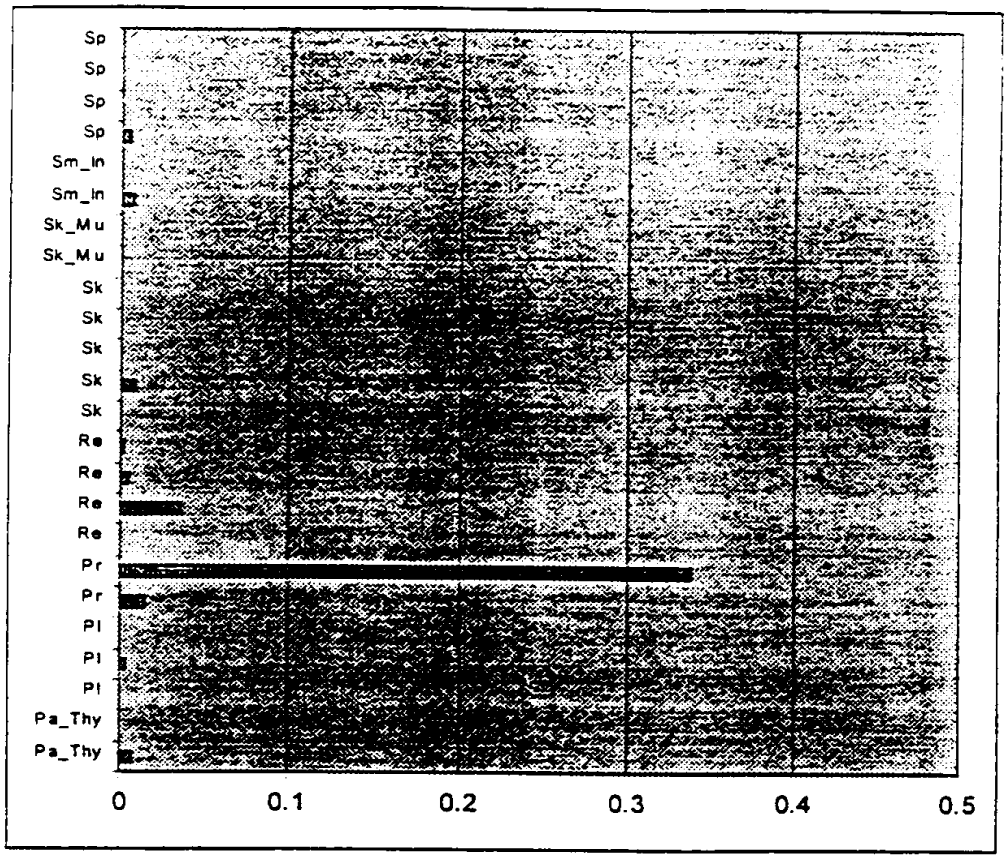


Figure 2D: Real-time PCR data of CASB618 expression in normal tissues (continued)

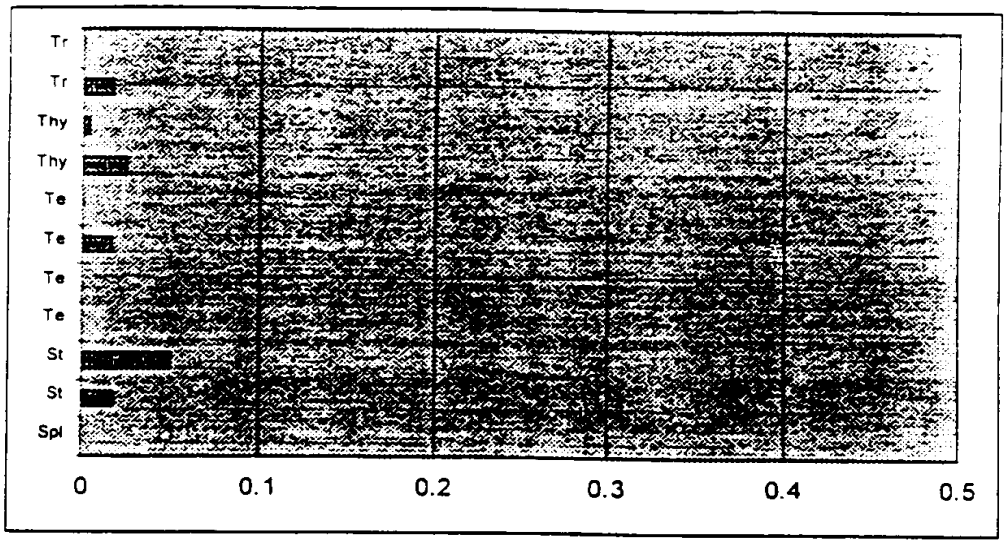


Figure 3: SDS-PAGE gel (12.5 %) of the *E. coli* AR120/pRIT15081 extract.

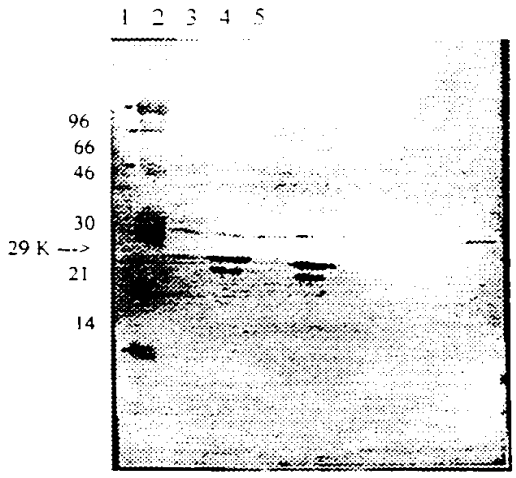
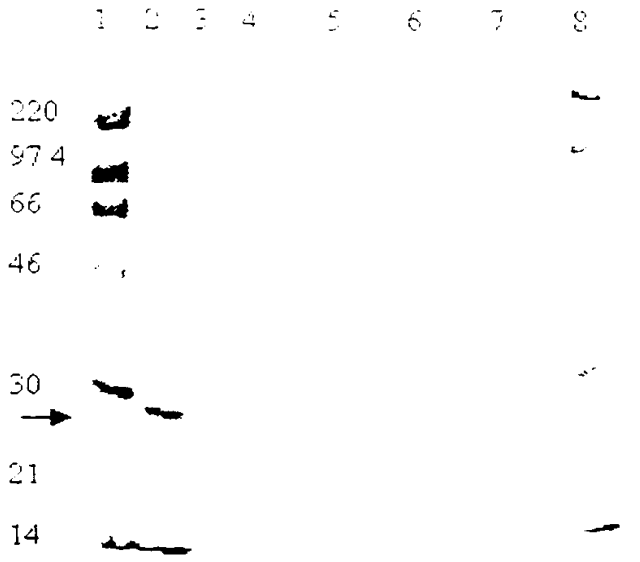
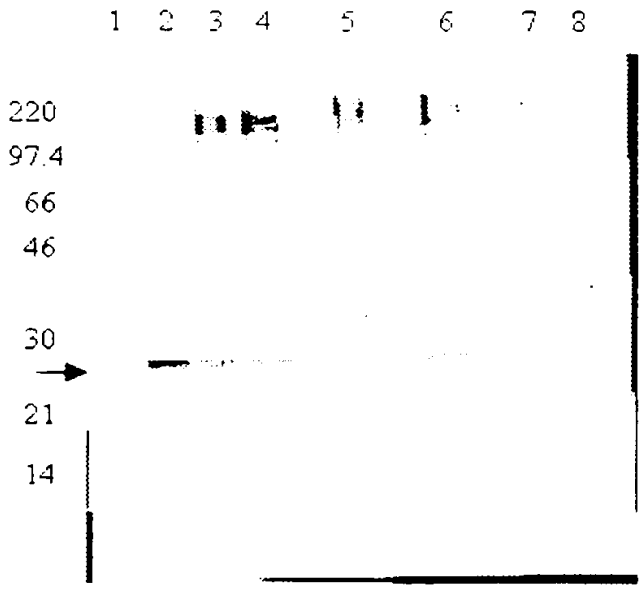


Figure 4: SDS-PAGE gels of purified CASB618



Coomassie 12%



WB revelated with Mab anti NS1

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(71) Applicant (*for all designated States except US*): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B–1330 Rixensart (BE).

(72) Inventors; and

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: NOVEL COMPOUNDS

(57) Abstract

CASB618 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing CASB618 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly ovarian and colon cancers, autoimmune diseases, and related conditions.

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(71) Applicant *(for all designated States except US)*: **SMITHKLINE BEECHAM BIOLOGICALS S.A.** [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventors; and

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(74) Agent: **DALTON, Marcus, Jonathan, William**; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

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WO 00/53748 A3

(54) Title: CASB618 POLYNUCLEOTIDES AND POLYPEPTIDES AND THEIR USE

(57) Abstract: CASB618 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing CASB618 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly ovarian and colon cancers, autoimmune diseases, and related conditions.

INTERNATIONAL SEARCH REPORT

Int Jonal Application No
PCT/EP 00/02048

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C12N15/75 C12N5/10 A61K38/17
C07K16/18 G01N33/68 G01N33/574 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"q149c11.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1875668 3' ....." EMBL SEQUENCE DATABASE, 23 November 1998 (1998-11-23), XP002147269 HEIDELBERG DE Ac AI274929 the whole document	5,8,12, 13
X	"qk58e08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE:1873190 3' ....." EMBL SEQUENCE DATABASE, 24 November 1998 (1998-11-24), XP002147270 HEIDELBERG DE Ac AI281211 the whole document	5,8,12, 13

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2000	02/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02048

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 32865 A (INCYTE PHARMA INC ;GOLI SURYA K (US); HILLMAN JENNIFER L (US)) 30 July 1998 (1998-07-30) abstract	14-16, 22,23, 28-31
A	WO 96 08514 A (ARLEN MYRON ;TSANG KWONG Y (US)) 21 March 1996 (1996-03-21) abstract; claims	17,22,28
P,A	WO 99 49030 A (BRUCK CLAUDINE ELVIRE MARIE ;SMITHKLINE BEECHAM BIOLOG (BE); COCHE) 30 September 1999 (1999-09-30) the whole document	1-25, 28-33
P,A	WO 99 49055 A (BRUCK CLAUDINE ELVIRE MARIE ;SMITHKLINE BEECHAM BIOLOG (BE); COCHE) 30 September 1999 (1999-09-30) the whole document	1-25, 28-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/02048

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9832865 A	30-07-1998	US 5840871 A	24-11-1998
		AU 6041998 A	18-08-1998
		EP 0975769 A	02-02-2000
WO 9608514 A	21-03-1996	US 5688657 A	18-11-1997
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		JP 10505749 T	09-06-1998
		ZA 9507634 A	27-05-1996
WO 9949030 A	30-09-1999	AU 3519199 A	18-10-1999
WO 9949055 A	30-09-1999	AU 3519299 A	18-10-1999

Claims

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to SEQ ID NO:2.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence of SEQ ID NO:2.

4. The isolated polypeptide of SEQ ID NO:2.

5. A polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of the immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:2

6. A polypeptide as claimed in any of claims 1 to 5 wherein said polypeptide is part of a larger fusion protein.

7. A polypeptide as claimed in any of claims 1 to 6 chemically conjugated to a carrier protein.

8. An isolated polynucleotide encoding a polypeptide as claimed in any of claims 1 to 6.

9. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

10. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

11. An isolated polynucleotide which comprises a nucleotide sequence which has at least 70% identity to that of SEQ ID NO:1 over the entire length of SEQ ID NO:1; or a nucleotide sequence complementary to said isolated polynucleotide.

12. The isolated polynucleotide as defined in any one of claims 8 to 11 in which the identity is at least 95%.

13. An isolated polynucleotide selected from:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(b) the polynucleotide of SEQ ID NO:1; and

(c) a polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof said polynucleotide encoding a protein which has similar immunogenic properties to those of the protein of sequence ID NO:2

or a nucleotide sequence complementary to said isolated polynucleotide

14. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 8 - 13.

15. A host cell comprising the expression vector of claim 14 or the isolated polynucleotide of claims 8 to 13.

16. A process for producing a polypeptide of claims 1 to 7 comprising culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A vaccine comprising an effective amount of the polypeptide of any one of claims 1 to 7 and a pharmaceutically acceptable carrier.

18. A vaccine comprising an effective amount of the polynucleotide of any one of claims 8 to 13 and a pharmaceutically effective carrier.

19. A vaccine comprising an effective amount of antigen presenting cells, modified by in vitro loading with a polypeptide of any one of claims 1 to 7, or genetically modified in vitro to express a polypeptide of claims 1 to 7 and a pharmaceutically effective carrier.

20. A vaccine as claimed in any one of claims 17 to 19 which additionally comprises a TH-1 inducing adjuvant.

21. A vaccine as claimed in claim 20 in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

22. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

23. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of any one of claims 1 to 5 which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the said polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any one of claims 1 to 7, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

24. A method for the treatment of a subject by immunoprophylaxis or therapy comprising *in vitro* induction of immune responses to a molecule of any one of claims 1 to 5, using *in vitro* incubation of the polypeptide of any one of claims 1 to 7 or the polynucleotide of any one of claims 8 to 13 with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for the treatment of disease.

25. A method as claimed in claim 24 wherein the treatment is for ovarian or colon cancer.

26. An agonist or antagonist to the polypeptide of claims 1 to 5.

27. A compound which is:
(a) an agonist or antagonist to the polypeptide of claims 1 to 5;
(b) isolated polynucleotide of claims 8 to 13; or
(c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of any one of claims 1 to 5;
for use in therapy.

28. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

29. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

30. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

31. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

32. An isolated polynucleotide selected from the group consisting of:
(a) an isolated polynucleotide comprising a nucleotide sequence which has at least 70% identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;
(b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3;
(c) the polynucleotide of SEQ ID NO:3.

33. A live vaccine composition comprising an expression vector or recombinant live micro-organism according to claim 14.